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POSTNATAL ANOXIA FROM CO_2 FOR ONE MINUTE PRODUCES SEX-SPECIFIC CHANGES IN CONTEXTUAL FEAR CONDITIONING IN ADULT RATS[1]

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Summary.—In a split-litter design, equal numbers of male and female rats were exposed either to carbon dioxide for 1 min. or to the control condition on either postnatal Days 9–10 or 17–18. At 120 days of age half the numbers of rats in each condition were given subclinical dosages of lithium (1.5 mEq/kg) and pilocarpine (15 mg/kg) or saline. 40 days later all rats were tested in a conditioned fear paradigm. A four-way analysis of variance as a function of the variables anoxia, age at the time of anoxia, sex, and subclinical seizure induction indicated a significant interaction between sex and anoxic history. *Post hoc* analysis showed that performance of male rats exposed to the anoxic condition did not differ from normal or anoxic female rats. The results suggest that only 1 min. of preweaning anoxia may produce permanent changes in contextual fear conditioning for male but not female rats.

Recently during neuropsychological assessments L. S. St-Pierre and M. A. Persinger were referred two preadolescent boys who had sustained a major episode of anoxia or hypoxia when they were about 4 years of age. Contrary to expectations, these two boys displayed intelligence and memory capacity that were more than two standard deviations above the age-expected mean. However, they had been referred because of their "emotional responsiveness" to social stimuli that had been interpreted as "social problems" by school personnel.

We decided to discern if brief anoxic episodes might enhance synaptic plasticity in the hippocampus. We selected fear conditioning in rats, a behavior strongly associated with the efficacy of long-term potentiation in the hippocampus (Stewart & McKay, 2000) to begin to test this hypothesis. The litters of six female albino Wistar rats (obtained from Charles River) were culled to eight pups (4 males; 4 females) within 48 hr. of birth. Half the numbers of male and female pups from each litter were removed from the nest and exposed in an airtight plastic container to a continuous flow of pure carbon dioxide for 1 min. The gas was delivered through the top of the container at 20 pounds per square inch. When they were removed visible respiration had ceased but returned within 30 sec. Spontaneous movement returned within 3 min.

Pups from three of the litters were exposed to the anoxia on postnatal Days 9 or 10, while the other three litters were exposed on postnatal Days 17 and 18. The remaining male and female pups from each litter were placed in the container but did not receive the treatment. We chose the first postnatal day period because the number of N-Methyl-D-Aspartate receptors that increases neurons' susceptibility to anoxic damage display a peak density within the hippocampus between Days 6 and 14 (Nyakas, Buwalda, & Luiten, 1996).

The litters were weaned at 21 days of age and placed in same sexed cages (3 rats/cage) according to standard protocols. When they were 120 days of age half the numbers of male and female rats from anoxic and control conditions were injected subcutaneously with 1.5

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mEq/kg of lithium chloride and then four hours later with 15 mg/kg of pilocarpine. The others received saline. Although this dosage does not induce overt seizures, we reasoned that the induction of subclinical seizures could affect the hippocampal formations of rats following a single episode of anoxia.

Forty days later (chosen for convenience) the rats ($n = 44$) were exposed to the fear-conditioning paradigm (Stewart & McKay, 2000) whereby after 3 min. of habituation to a setting three unsignalled 2-sec., .5-mA footshocks were delivered at 60-sec. intervals. The numbers of freezing (no movements) every 7 sec. following these shocks (21 observations) were recorded. The percentage of freezing behavior 24 hr. later in same setting during 60 equally spaced (every 8 sec.) observations during an 8-min. period was recorded for each rat. Four-way analysis of variance (anoxia vs control; subclinical seizures vs saline; 9–10 days vs 17–18 days at time of anoxia; sex) indicated no significant main effects for the treatments (all dfs = 1,32, $p > .05$). Although the males exhibited ($F_{1,33} = 6.90$, $p < .05$) more immobility ($M = 42.7\%$, $SEM = 3.9\%$) than did the females ($M = 29.2\%$, $SEM = 2.7\%$), there was a statistically significant ($F_{1,33} = 9.17$, $p < .01$) interaction between sex and anoxic condition. In comparison there were no significant main effects or interactions for the percentage of numbers of postshock freezing during the pairing (first) day. The means and *SEM*s were 39.7% and 5.0% for the males and 37.5% and 2.9% for the females.

The means and standard errors of the mean for the percentage of numbers of nonmovements during the 8 min. to context during which the rats had been shocked the previous day was for control females ($n = 14$): 51.2 and 4.6, anoxic females ($n = 10$): 45.3 and 9.0, control males ($n = 10$): 86.8 and 5.4, and anoxic males ($n = 10$): 55.3 and 9.8. *Post hoc* analyses by Tukey test ($p < .05$) indicated that the source of the interaction was the greater proportion of freezing responses for the control males than for all other groups which did not differ from each other.

Male rats exhibit more robust long-term potentiation within the hippocampus following stimulation of the perforant pathway than do female rats (Maren, De Oca, & Fanselow, 1994) and stronger responses during a contextual fear paradigm than do female rats. These results indicate that the early anoxia reduced the stronger responses typical of the normal male hippocampus such that the contextual fear in conditioning was similar to that of normal females. Although we assumed that anoxia was the operative mechanism, the excessive concentration of carbon dioxide may have permanently affected the activity or viability of cells sensitive to carbonic anhydrase. If comparable changes occur within the human brain then very brief periods of anoxia during early development, perhaps not even discernable to the parents, may be sufficient to affect the lability of hippocampal function in the adult.

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